Amendment to the specification:

Please amend the Abstract in the following manner:

A method of detecting Parkinson's disease through MRI of substantial nigra pars ~~compacts~~ compacta (SNc) tissue. The method involves obtaining a gray matter suppressed (GMS) MRI signal from the SNc tissue, obtaining a white matter suppressed (WMS) MRI signal of the SNc tissue, and combining information from the GMS and WMS MRI signals to produce resultant signals indicative of Parkinson's disease. A similar method can be used to detect Progressive Supranuclear Palsy. A method of distinguishing between the two diseases involves obtaining at least two starting MRI images of SNc tissue using different MRI parameters, and combining the starting images to compute resultant signals differentiating between the two forms of parkinsonism.

Please amend the paragraph at page 2, lines 4-10 in the following manner:

The two starting images for an MRI slice can be two images that include the substantia nigra pars ~~compacts~~ compacta (SNc)--a grey matter suppressed (GMS) MRI image and a white matter suppressed (WMS) image. A ratio of the two images produces a ratio image. A ratio of two regions of interest (ROI), one from the medial segment and one from the lateral segment of the SNc for each slice, and combining the measures for two or more slices, gives numerical values indicative of the presence and/or staging of Parkinson's disease and the presence of

Progressive Supranuclear Palsy.

Please insert the following on page 2 after line 23:

Fig. 4 is a copy of Fig. A in article (1) cited in this specification. The Upper row shows upper and lower ratio images of a normal object. Note that the substania nigra pars compacta (SNc) reaches the edge of the peduncle in the upper slice and becomes smaller in the lower slice. The substania nigra pars reticulata (SNR) is also seen in the upper slice, extending into the corticospinal tracts anteriorly. The colour bar shows the psuedocolour used for display and ranges from 0 to 225 (bottom to top). The ratio image of an early case shows, in the upper slice, thinning and loss of signal in the lateral part of the SNc. Note that the lower slice shows islands of destruction. The ratio images of an advanced stage show considerable signal loss in the SNc in both upper and lower slices. In addition, the SNc is essentially reduced to two rings of preservation in the lower slice.

Fig. 5 is a copy of Fig. B in article (1) cited in this specification. The graph is a plot of DU and DL (see text) for patients and controls. Note that the controls (green dots) cluster at the origin and that the patients (red dots) are distributed along a diagonal path in correspondence with Hoehn and Yahr Disease stage (indicated by Roman numeral next to each dot).

Fig. 6 is a copy of Fig. 1 in article (2) cited in this specification. Upper rows displays an example of axial WMS and GMS MR acquisition images of the mesencephalon in a control participant. The cerebral peduncle (second row, left) extracted from the WMS midbrain image serves as a template to extract the GMS image of the cerebral the cerebral peduncle shown on the right. The SNc is seen as bright arch in the peduncular WMS image, whereas it appears as a dark band in the corresponding GMS image. Note also the substantia nigra pars reticula (SNR)

reaching across the crus cerebi toward the SNc. The raio image (WMS/GMS) of the two images in the second row yields the color-coded ratio image displayed on the bottom. All black and white images are shown using a standard display of 256 gray levels. The color image uses a 256-pseudocolour lookup table.

Fig. 7 is a copy of Fig. 3in article (2) cited in this specification. Radiologic indices are displayed for the six control participants and the six patients with Parkinson's disease. The is no overlap between the groups, which are distinct by Student's t Test ($P < .00005$). The error bars represent one SD.

Fig. 8 is a copy of Fig. 4 in article (2) cited in this specification. Unified Parkinson's Disease Rating Scale scores for the six patients ranging from 12 to 71 are plotted versus radiologic indices. A linear regression analysis was conducted, yielding a linear correlation coefficient of $r = 0.99$.

Please amend the paragraph at page 2, lines 24-28 continued on page 3, lines 1-2 in the following manner:

As described in the two articles cited below and hereby incorporated by reference herein (and also reproduced below in this Detailed Description), the possibility of detecting Parkinson's disease using MRI has been a long-sough goal: (1) Hutchinson M, Raff U, Parkinson's disease: a novel MRI method for determining structural changes in the substantia nigra. J Neurol Neurosurg Psychiatry December 1999; 67:815-818; and (2) Hutchinson M, Raff U, Structural Changes of the Substantia Nigra in Parkinson's Disease as Revealed by MR Imaging, AJNR Am J Neuroradiol 21:697-701, April 2000.

Please amend the paragraph at page 3, lines 3-11 in the following manner:

In preferred embodiments described herein and in the two articles, this goal is met by using imaging that enhances changes in a brain area that are believed to be indicative of Parkinson's disease. Using a combination of two MRI imaging inversion-recovery sequences, the ~~substantial~~ substantia nigra is imaged and a radiologic index is derived and used to quantify changes in a manner enabling detection even in asymptomatic patients and also enabling objective staging of the disease. Structural changes in the substantia nigra, mainly in the pars compacta (SNc), are highlighted using the preferred MRI signals and processing, and numerical scored are derived to indicate the presence and/or staging of the disease.

Please amend the paragraph at page 3, lines 12-29 continued on page 4, lines 1-6 in the following manner:

In a first method, described in detail in article (1), which is reproduced below in this Detailed Description ~~and, therefore, not repeated here~~, a white matter suppressed (WMS) image and a grey matter suppressed (GMS) images are obtained, using MRI inversion-recovery pulse sequences with the parameters stated in article (1) for the indicated MRI scanner, or using other sequences or parameters or MRI scanners that produce WMS and GMS MRI images differing from each other in a manner allowing for processing that highlights changes in the SNc associated with Parkinson's disease. As described in article (1), it has been found that the GMS signal tends to increase in SNc areas affected by the disease while the WMS signal tends to

decrease in the same areas. A ratio image of the WMS to GMA MRI images of an MRI sclice tends to have an increased sensitivity to changes in the substantia nigra due to Parkinson's disease than either of the GMS and WMS images alone. A numerical measure can be obtained, for example by taking a ratio of a medialto-lateral regions of interest (ROI) in the substantia nigra imaged in each MRI slice. Each ROI can be about 200 pixels in size, although different sizes can be used, and this can also depend on the pixel resolution of the image. If the substantia nigra is imaged in two slices, an upper slice and a lower slice, a total of four ROI are defined. A ratio RU is computed of the pixel values of the lateral to the medial ROI in the upper slice, and a similar ration RL is computed for the lower slice. The resulting ratio values are further processed as described in article (1) to obtain a pair of numerical measures DU and DL. In a plot of the type illustrated in FIG. A of article (1), the numerical measures DU and DL give points that at in a cluster for Parkinson's disease patients that is well spaced from a cluster for patients without the disease, and also are at different positions for different stages of the disease, thus enabling detection and staging of the disease.

Please amend the paragraph at page 4, lines 6-11 in the following manner:

For TR much greater than TE, the ratio image depends only or mainly on T1, so the signal values of the ratio image can be recast in the form of a T1 map. This is so because for IR pulse sequences the pixel value P(x,y) at a pixel position (x,y) can be ~~H0~~ expressed as the value of T1 at the same position (x,y), thus creating a T1 map. Such a ~~T1~~ T1 map can be similarly analyzed to compute similar numerical measures that highlight the presence and staging of Parkinson's disease.

Please amend the paragraph at page 4, lines 11-17 in the following manner:

In another embodiment, described in detail in article (2), which is reproduced below in this Detailed Description ~~and, therefore, not repeated here~~, WMS and GMA MRI signals are similarly obtained but are processed differently, to compute a numerical radiologic index or score RI that is similarly useful for both detecting and staging Parkinson's disease, as illustrated at FIGS. 3 and 4 of article (2).

Please amend the paragraph at page 4, lines 27-29 and page 5, line 1 in the following manner:

FIG. 3 illustrates that the WMS and GMS images discussed above and in ~~articled~~ articles (1) and (2) can b e used to provide indications of Progressive Supranuclear Palsy (PSP). As explained in the caption of the figure, the changes that are visualized allow distinguishing between the two forms of parkinsonism radiographically.

Please insert the following on page 5 after line 1:

Article (1) cited above, is reproduced below. Figs. A and B of the article are added to this specification as Figs. 4 and 5, respectively:

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SHORT REPORT

Parkinson's disease: a novel MRI method for determining structural changes in the substantia nigra

Michael Hutchinson, Ulrich Raff

Abstract

***Objectives*—To use MRI in a novel way to image and quantify the changes occurring in the substantia nigra in Parkinson's disease.**

***Methods*—Six patients with idiopathic Parkinson's disease were compared with six age matched control subjects. The subjects were imaged using a combination of pulse sequences hypothesised to be sensitive to cell loss.**

***Results*—The images showed patterns of change in patients with Parkinson's disease. Highly significant differences between the patients and control population were found ($p<0.001$).**

***Conclusions*—This methodology suggests the possibility of detecting presymptomatic disease in those judged to be at risk, and also in confirming the diagnosis in patients with early disease. Furthermore, the technique seems to hold promise as a means for staging the disease, and possibly differentiating other forms of parkinsonism.**

J Neurol Neurosurg Psychiatry 1999;67:815–818)

Keywords: Parkinson's disease; substantia nigra; magnetic resonance imaging

Parkinson's disease involves the degeneration of neurons in the substantia nigra (mainly in the pars compacta). About 5% to 10% of all cases may be familial and inherited in an autosomal dominant pattern.[1] In the era of potential neuroprotective therapies for this disorder, the earliest diagnosis and perhaps even the detection of presymptomatic disease is highly desirable. Moreover, a simple non-invasive method for staging the disease is of importance in evaluating the results of neuroprotective interventions.

The possibility of detecting structural changes in the substantia nigra pars compacta (SN_c) using conventional MRI has the advantage of a simple and readily available, relatively inexpensive modality for diagnosing and studying the disease. Several previous publications have employed various MRI strategies to demonstrate nigral changes using MRI. These fall essentially into two categories. The first involves using pulse sequences sensitive to the increased iron deposition in the substantia nigra that is seen in Parkinson's disease.[2–4] Iron is deposited in the nigra in normal aging, however, which may create difficulties in separating patients from controls, particularly in the elderly population.[5] The second approach involves measurement of the width of the pars compacta of the substantia nigra[6–8] using T2 weighted images. Although thinning of this structure does occur in Parkinson's disease, there may be considerable variation of the thickness of this structure in patients and even in normal subjects. Moreover, the width itself may be difficult to define with precision.

At the present time, the most sensitive imaging techniques for the detection of Parkinson's disease are positron emission tomography (PET)[9–11] and single photon emission computed tomography (SPECT).[12] Both techniques measure changes in the striatum but not in the substantia nigra itself. PET has traditionally used a label of striatal uptake of DOPA, whereas SPECT utilises a tracer (β-CIT), which is a label for dopamine transporters in the striatum.

We present here a novel MRI technique for imaging the structural changes in the substantia nigra itself, using a combination of two pulse sequences, and taking advantage of the known geometric variation in the degeneration of this nucleus (from lateral to medial and rostral to caudal).[13] The sequences are both of the inversion recovery type, one designed to suppress white matter and the other to suppress grey matter. By combining these pulse sequences we have been able to demonstrate changes in the substantia nigra in even the earliest cases of symptomatic disease, demonstrating that these cases are quite distinct from normal subjects. This suggests the possibility of detecting presymptomatic disease. Furthermore, our results also suggest the possibility of staging the disease.

Patients and methods

SUBJECTS

Six patients in various stages of the disease (Hoehn and Yahr stages I to III, ages 38 to 70, mean age 58) were scanned. All patients were taking their usual antiParkinson medication at

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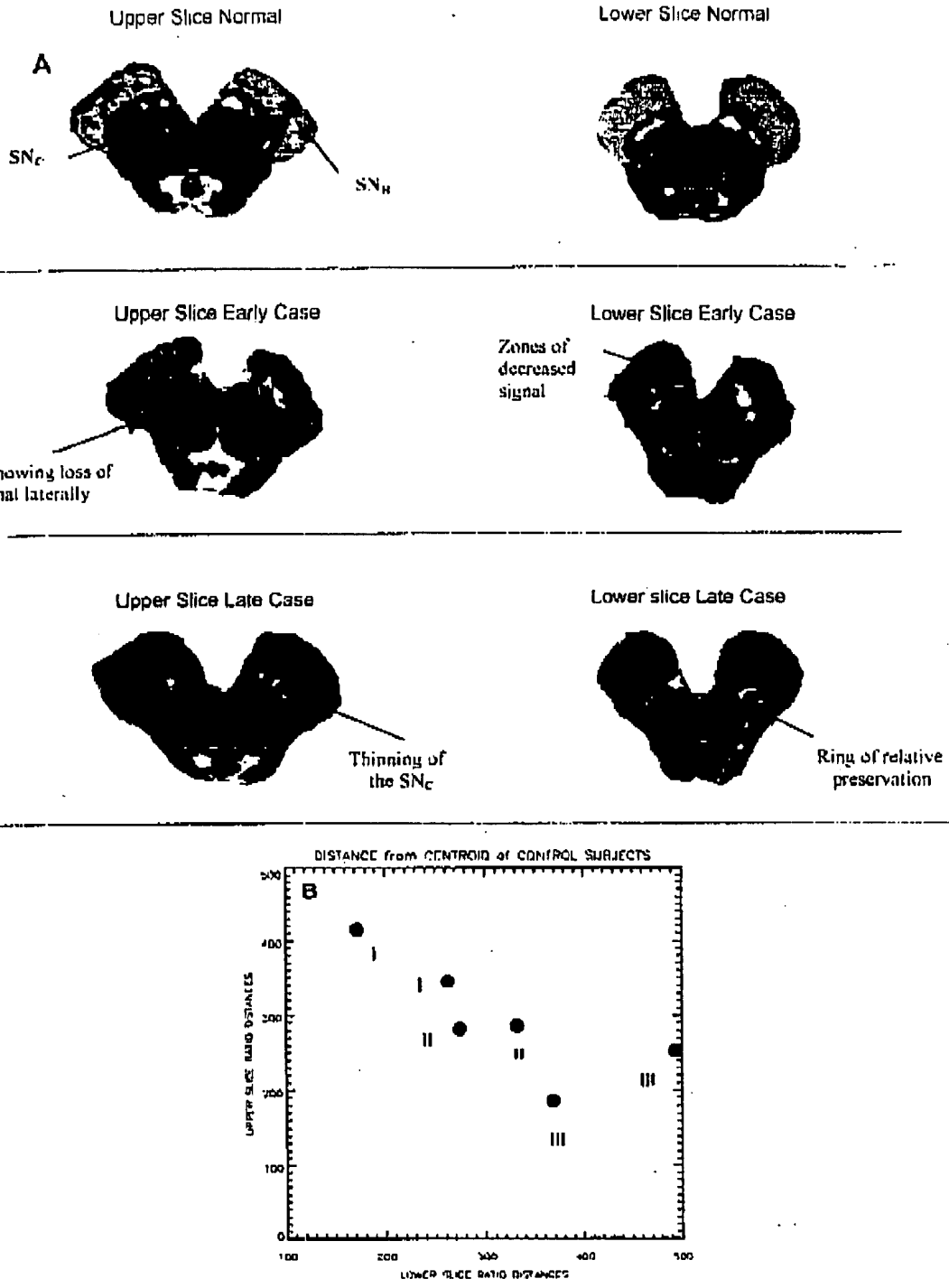

(A) The upper row shows upper and lower ratio images of a normal subject. Note that the substantia nigra pars compacta (SN_c) reaches the edge of the peduncle in the upper slice and becomes smaller in the lower slice. The substantia nigra pars reticulata (SN_R) is also seen in the upper slice, extending into the corticospinal tracts anteriorly. The colour bar shows the pseudocolour used for display and ranges from 0 to 255 (bottom to top). The ratio image of an early case shows, in the upper slice, thinning and loss of signal in the lateral part of the SN_c. Note that the lower slice shows islands of destruction. The ratio images of an advanced stage show considerable signal loss in the SN_c in both upper and lower slices. In addition, the SN_c is essentially reduced to two rings of preservation in the lower slice. (B) The graph is a plot of DU and DL (see text) for patients and controls. Note that the controls (green dots) cluster at the origin and that the patients (red dots) are distributed along a diagonal path in correspondence with Hoehn and Yahr disease stage (indicated by a Roman numeral next to each dot).

the time of the scan. Six normal subjects (ages 37 to 72, mean age 56) were also scanned using

son's disease, and all were examined and questioned by an experienced neurologist to rule

 of early

METHODS

Scanning was performed using a Siemens 1.5 T Vision system. To avoid head motion artefacts, all subjects were immobilised using a chinstrap, which had proved invaluable in a prior functional MRI study,[11] where head immobilisation was perhaps even more crucial. White matter suppressed (WMS) images were obtained with the following pulse sequence: inversion recovery (modulus), TE=20 ms, TI=250 ms, and TR=1450 ms. Grey matter suppressed (GMS) images were obtained with a similar inversion recovery sequence but with the following parameters: TE=20 ms, TI=420 ms, and TR=2000 ms. The field of view was 200 mm and the image matrix was 256×256 (NEX=2). Slice thickness was 3 mm and the gap was 0.2 mm. Four axial slices were obtained in each case, with slice selection being obtained from a sagittal scout image of the brain stem. Slices were chosen perpendicular to the longitudinal axis of the midbrain. Using the WMS image it was ascertained that the substantia nigra was visualised in each of the four slices. By selecting the middle two slices for analysis we thereby ensured that there was no contamination by volume averaging.

Changes in signal were seen in both WMS and GMS images, the GMS signal increasing in areas of degeneration, whereas the WMS signal decreased in the same areas. Therefore, for each of the selected slices, a ratio of WMS to GMS images was generated, this ratio image providing increased sensitivity compared with either the WMS or GMS alone. Nevertheless changes in the WMS images were less substantial than those seen in the GMS images, so that the WMS image served to define the boundaries of the substantia nigra, whereas the GMS image (in patients) did not. A blinded observer then performed a region of interest (ROI) analysis, with regions of about 200 pixels being placed within the boundaries of the substantia nigra defined in both lateral and medial segments of the WMS image. These same regions were then placed on the ratio images and average pixel values for the lateral and medial segments computed. Because of uncertainties in absolute signal levels it is common practice in quantifying MRI to take ratios within each subject to make valid a comparison between subjects. Therefore, for each subject the ratio R of lateral to medial values was defined, both for the upper slice (RU) and for the lower slice (RL). Furthermore, these values were divided into a ratio for the left SN_c, denoted by subscript "l", and the right SN_c, denoted by subscript "r". Therefore each subject in the study was represented by two pairs of values (RU_l, RU_r) and (RL_l, RL_r), the first pair representing the upper slice and the second the lower. The centroid (the mean value of the ratio) of these values for normal subjects was defined as RU and RL. These values were also defined for both left and right SN_c. For each subject (both patients and normal controls) the distance from this centroid was defined as the pair of values (DU, DL), where:

$$DU = \sqrt{(RU_l-\overline{RU_l})^2+(RU_r-\overline{RU_r})^2}$$

$$DL = \sqrt{(RL_l-\overline{RL_l})^2+(RL_r-\overline{RL_r})^2}$$

For all 12 subjects, these (euclidean) distances (DU and DL) are presented in figure B.

Results

Figure A shows the ratio images of the upper and lower slices for a typical normal subject, an early case (Hoehn and Yahr stage I), and a more advanced case (Hoehn and Yahr stage III) respectively. These are displayed in pseudocolour to enhance the visual representation. In normal subjects it is seen that the substantia nigra extends to the lateral borders of the peduncle in the upper but not in the lower slice. This is demonstrated in the WMS image (not shown). In the graph, each subject is represented by a pair of values DU and DL, as defined in the Methods section. Normal subject values are represented as green solid circles while the patients' values are displayed as red solid circles. Next to each patient symbol is a Roman numeral denoting the Hoehn and Yahr stage of the patient (as judged off medication). A two tailed *t* test separated normal subjects from patients ($p<0.001$).

Discussion

The neuroimaging of the substantia nigra in Parkinson's disease, by means of conventional MRI techniques, has been a desirable but elusive goal. Potential benefits include the detection of presymptomatic disease and the staging of the disease. Detection of presymptomatic disease, especially in the inherited disorder, could allow the early introduction of neuroprotective treatments in those determined to be at risk. Furthermore the potential for staging the disease would allow for evaluation of neuroprotective interventions in the symptomatic patient. Techniques of this kind may also serve to differentiate idiopathic Parkinson's disease from other forms of parkinsonism.

Two inversion recovery sequences were used and a ratio image defined from them. We note that the ratio image depends (for TR much greater than TE) only on T1, so that the signal values in the ratio image could be recast in the form of a T1 map. To define a numerical measure of degeneration (DU and DL) which could be used to make a comparison between subjects, intersubject variability in absolute signal was eliminated by making each subject his own control and defining a lateral to medial ratio of signal intensity within this ratio image. We note that the numerical measure is therefore defined in terms of a ratio of ratios.

Visual inspection of the images confirms that the substantia nigra degenerates from lateral to medial and in a rostral to caudal direction. There is also thinning, and the structure takes on a mottled appearance compared with the normal subjects. In particular, we noted that in all six patients, in the lower slice there were zones of cell loss surrounded by rings of relative preservation. These were not seen in any of the normal subjects. The significance of this pattern of cell loss has yet to be ascertained. However, the sharp delineation of these structures serves to demonstrate that the overall changes seen in the patient group were not the result of motion

artefact. Artefacts of this kind tend to blur and degrade small structural changes.

The graph shows that the normal subjects are clustered close to the origin of the plot. The least symptomatic patient, a 38 year old woman with a 1 year history of the disease is seen in the top left hand corner. The clear separation of this patient from the group of normal subjects suggests the possibility of detecting presymptomatic disease. Moreover, the graph also suggests the possibility of staging the progression of the disease, as there seems to be a correlation between severity of disease and the distance from the top left hand corner. Note that DU tends to normalise as the disease progresses. This reflects the fact that the lateral segment reaches an asymptotic signal value first, and the medial segment finally approaches the same asymptote, so that lateral and medial segments finally tend to the same value.

What is demonstrated here for the first time is the potential efficacy of inversion recovery sequences in imaging the substantia nigra in both health and disease. It is likely that different pulse sequence parameters can be found that will yield further improvements in image quality. Moreover, further work will be needed to refine the technique, particularly in the use of thinner slices, faster sequences, and the use of automated image segmentation techniques.

This study was funded in part by the Myra Fox and Max Smedresman funds for research into Parkinson's disease.

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Article (2) cited above, is reproduced below. Fig. 1 of the article is added to this specification as Fig. 6. Fig. 2 of the article already appears in this specification as Fig. 1. Figs. 3 and 4 of the articles are added to this specification as Figs. 7 and 8, respectively.

AJNR ... J Neuroradiol 21:697–701, April 2000

Structural Changes of the Substantia Nigra in Parkinson's Disease as Revealed by MR Imaging

Michael Hutchinson and Ulrich Raff

BACKGROUND AND PURPOSE: The possibility of using MR imaging as a sensitive marker of the structural changes in Parkinson's disease has been a long-sought goal. We describe a new method for imaging and quantifying the morphologic changes of the substantia nigra in Parkinson's disease and compare radiologic findings with clinical evaluation.

METHODS: Using a combination of two MR imaging inversion-recovery pulse sequences, the substantia nigra was imaged in six patients with Parkinson's disease and six age-related control participants. A radiologic index was defined and used to quantify the signal changes that were observed in the patients. The radiologic index was compared with clinical scores obtained from the Unified Parkinson's Disease Rating Scale.

RESULTS: The images showed loss of signal in a lateral-to-medial gradient in cases of Parkinson's disease, corresponding to the known neuropathologic pattern of degeneration. The radiologic index was highly correlated with the Unified Parkinson's Disease Rating Scale score, and there was no overlap in radiologic indices between the patient and the control groups ($P < .00005$).

CONCLUSION: This study suggests that MR imaging is sensitive to structural changes in even the earliest cases of Parkinson's disease, thereby indicating the potential for detecting presymptomatic disease. Furthermore, a radiologic measure has been defined that correlates with the conventional clinical measure of disease severity. Therefore, MR imaging could prove to be a sensitive biological marker for objective staging of the disease.

Parkinson's disease is a common, progressive neurodegenerative condition involving mainly the degeneration of neurons in the substantia nigra pars compacta (SN_C). Approximately 10% to 20% of all cases may be familial and inherited in an autosomal dominant pattern with incomplete penetrance (1). There is general consensus that at the onset of symptoms, the majority of neurons in the SN_C have degenerated. Although surgical and medical interventions can alleviate the symptoms for many years, they probably do not alter the rate of degeneration. Nevertheless, putative neuroprotective interventions aimed at slowing or even halting disease progression are currently under investigation (2, 3). Therefore, considering the enormous human and socioeconomic costs of Parkinson's disease, the earliest detection, and even the detection of presymptomatic disease, is highly desirable. Furthermore, an objective radiologic measure would be of value for assessing the effects of such interventions in both asymptomatic and symptomatic patients.

Clinical staging is the present standard of reference for following disease progression. The most comprehensive and widely used scale is the Unified Parkinson's Disease Rating Scale (4). Despite its strengths, however, the scale has a number of distinct limitations. Patients have symptoms that fluctuate from day to day, and there may be considerable interobserver variability. These sources of error alone require that clinical trials of drug efficacy must include a large number of participants. Also, by definition, the scale renders one incapable of detecting presymptomatic disease.

At the present time, the most sensitive imaging techniques for the detection of Parkinson's disease are positron emission tomography (5–8) and single-photon emission CT (9–11). Both techniques measure striatal changes, but not changes in the SN_C itself. Positron emission tomography traditionally measures the uptake of dopa in the striatum, whereas single-photon emission CT uses a tracer, β-CIT, which is a label for dopamine transporters. Both techniques are sensitive to disease stage in symptomatic patients and hold the promise for revealing presymptomatic disease. They are, however, rela-

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tively costly and not widely available. Moreover, both techniques require that a patient be off medication for at least 12 hours before the study. This can be uncomfortable for a patient with advanced symptoms, and is potentially dangerous.

On the other hand, MR imaging is a simple and relatively inexpensive technique that is widely available. Its use in this context has therefore been a highly desirable but elusive goal. Various efforts have been made to show nigral changes by using MR imaging. These fall essentially into two categories. The first category is based on the changes in T2 and T2* signal associated with the increased iron deposition in the SN_C occurring in cases of Parkinson's disease (12–14). Iron is deposited in the SN_C in normal aging, however, and this may create difficulties in separating patients from control participants. In particular, in a large study, changes in T2-weighted imaging findings did not correlate either with disease duration or with clinical severity (14). The second category involves measurement of the width of the SN_C (15–17) by using T2-weighted images. Although thinning of this structure does occur in cases of Parkinson's disease, the nucleus is only a few millimeters wide and takes on an irregular appearance as the disease progresses. This makes the width difficult to define with precision.

We have recently developed a different MR imaging approach for evaluating the SN_C, which uses a combination of two pulse sequences, each of the inversion-recovery types, which are therefore heavily T1-weighted. This approach was based on the hypothesis that T1-weighted imaging depends mainly on the intracellular space and that such sequences ought to be sensitive to the changes in intracellular volume occurring with cell death.

One sequence was specifically designed to suppress peduncular white matter and the other to suppress nigral gray matter. These image types are white matter–suppressed (WMS) and gray matter–suppressed (GMS). A combination of these pulse sequences showed structural changes in the substantia nigra in even the earliest cases of symptomatic disease.

Methods

Patients

Six patients in various stages of the disease (Unified Parkinson's Disease Rating Scale scores, 12–71; age range, 38–70 years; mean age, 58 years) and six age-matched, healthy control participants (age range, 37–72 years; mean age, 56 years) underwent imaging. Informed consent was obtained from patients before imaging was performed. All patients were taking their usual anti-Parkinson's disease medication at the time of the imaging. None of the control participants had any known relatives with Parkinson's disease, and all were examined and questioned by an experienced neurologist to rule out signs and symptoms suggestive of early Parkinson's disease.

MR Imaging

Imaging was performed using a Siemens 1.5-T Vision system. To avoid head motion artifacts due to respiration, all patients and participants were immobilized in flexion-extension of the neck by means of a chin strap.

Imaging of the SN_C was performed using two distinct inversion-recovery sequences. The first of these was designed to suppress nigral gray matter, whereas the second was designed to suppress the white matter of the crus cerebri. As previously hypothesized, for patients, the GMS images showed large variations in signal intensity within the SN_C. These signal changes were particularly pronounced at the anterior and lateral edges, making the boundaries of this structure difficult to define in patients with advanced disease. On the other hand, signal variation in the WMS images was less than but in a direction opposite that of the GMS images. In WMS images, the edges of the SN_C were distinct, even in patients with advanced disease. Therefore, by taking the ratio of the two images (WMS/GMS), not only is contrast improved (because the signal changes are additive) but the borders of the SN_C are defined, even in advanced cases. For each pixel in the ratio image, a signal intensity ratio is defined as its value in the WMS image divided by its value in the GMS image.

WMS images were obtained with an inversion-recovery (modulus) pulse sequence of 1450/20 (TR/TE) with an inversion time of 250 ms. GMS images were obtained with a similar inversion-recovery sequence but with parameters of 2000/20 and an inversion time of 420 ms. The field of view was 200 mm, and the image matrix was 256 × 256. The number of acquisitions was two. The section thickness was 3 mm with a gap of 0.2 mm. Four axial sections were obtained in each case, with sections selected from a sagittal scout image of the brain stem. Sections were chosen to be perpendicular to the longitudinal axis of the mesencephalon. The lowest section was taken just at the level of the most rostral part of the pons. Using the WMS image, it was ascertained that the SN_C was visible in each of the four sections. By selecting the middle two sections for analysis, we ensured that there was no contamination by volume averaging.

Data Analysis

The WMS and GMS axial images were used to extract the mesencephalic peduncular structures. Figure 1 shows the original WMS and GMS images obtained through the midbrain in a control participant. A manual region-of-interest technique applied to the WMS images was used to extract simultaneously the cerebral peduncle in WMS and GMS images from adjacent structures (Fig 1, *second row*). Note the improved contrast in the cerebral peduncle is because signal intensities in the WMS image are redisplayed in 256 gray levels whereas, on the contrary, the corticospinal tracts correspond to high intensities in the GMS image. The ratio image (WMS/GMS) of the cerebral peduncle was then computed and displayed in color by using a 256-pseudocolor lookup table.

To obtain a numerical value for the degree of signal change in the SN_C, we took advantage of the known geometric variation in the degeneration of this nucleus (ie, from lateral to medial) (18). All analyses were conducted on the middle two sections of the ratio images by an observer blinded to whether the image was of a patient or a control participant. All images were identified to this observer only as a number. Regions of interest (approximately 200 pixels each) were placed on the lateral and medial segments of the SN_C. For each patient and participant in the study, this ratio was computed for both right and left sides of each of the two sections and the lateral and medial sections of the SN_C. The mean pixel value within each region of interest was then used to define the ratio of pixel values in all four values averaged together to give R_{AV}. As expected, the R_{AV} of control participants all had values close

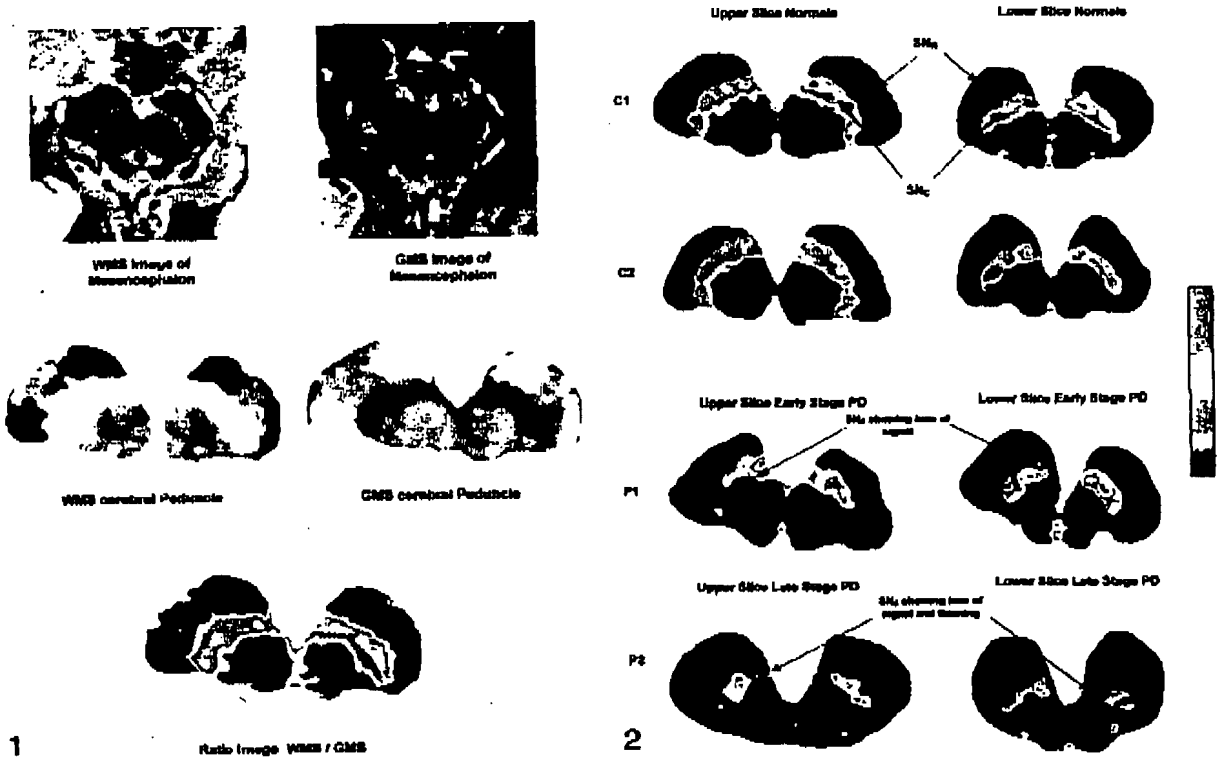


Fig 1. *Upper row* displays an example of axial WMS and GMS MR acquisition images of the mesencephalon in a control participant. The cerebral peduncle (*second row, left*) extracted from the WMS midbrain image serves as a template to extract the GMS image of the cerebral peduncle shown on the *right*. The SN_C is seen as a bright arch in the peduncular WMS image, whereas it appears as a dark band in the corresponding GMS image. Note also the substantia nigra pars reticulata (SN_R) reaching across the crus cerebri toward the SN_C. The ratio image (WMS/GMS) of the two images in the *second row* yields the color-coded ratio image displayed on the *bottom*. All black and white images are shown using a standard display of 256 gray levels. The color image uses a 256-pseudocolor lookup table.

Fig 2. Ratio images of the cerebral peduncle displayed in pseudocolors show the morphologic characteristics of the SN_C in two control participants (C1 and C2) and the structural changes in two patients with Parkinson's disease (P1 and P2). The substantia nigra pars reticulata (SN_R) is indicated for participant C1. Notice that the SN_C in control participants reaches out toward the peduncular edge in the upper section taking on the form of an arch. In the images of patient P1, who has Parkinson's disease, thinning and loss of signal can be seen in the lateral segment of the SN_C in the upper section. The lower section shows islands of cell loss on both sides of the SN_C. Note the considerable thinning and loss of signal in both upper and lower sections of the images of patient P2, who has late-stage Parkinson's disease. *Left* and *right sides* show two rims of preserved signal.

to one, whereas the patients had a wide range of values. A radiologic index was then defined as follows:

$$RI = 100\,(1 - R_{AV})$$

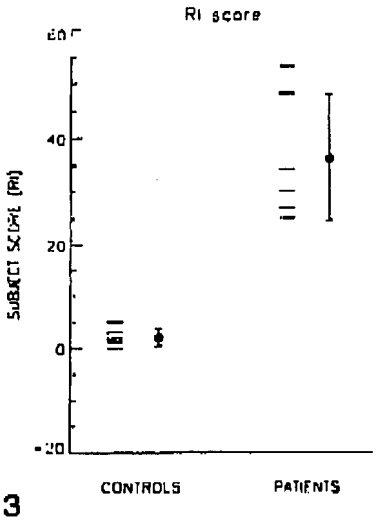
Results

Because the eye is more sensitive to color than to gray scale, rendering the images in pseudocolor enhances the structural changes (Fig 2). This is achieved using a 256-level "rainbow" color table, as shown at the side of Figure 2. Figure 2 shows the SN_C of two control participants (C1 and C2) and two patients (P1 and P2). Note the presence of the substantia nigra pars reticulata in all cases reaching from the posterior SN_C across the crus cerebri toward the edge of the cerebral peduncle. The patient with early-stage Parkinson's disease (P1) shows lateral thinning and loss of signal in the SN_C. The lower section of the same patient shows islands of signal loss. Similar areas of signal loss were observed in the SN_C of all patients. The last row displays upper and lower sections for a patient with advanced-stage Parkinson's disease (P2). Note particularly the considerable signal loss and thinning of the SN_C in the medial segments of the upper section. The lower section shows severe thinning as well as two rims of relatively preserved signal.

Two observers evaluated the data. An experienced neurologist, blinded to the computation of the radiologic index, scored each patient by using the Unified Parkinson's Disease Rating Scale (2). The second observer, blinded to the identity of each participant, evaluated the radiologic indices for all participants. No interobserver reliability assessment was performed. Figure 3 shows that there is no overlap between patients and control participants and that the two groups are distinct ($P < .00005$, Student's t test). Figure 4 shows a high correlation

FIG 3. Radiologic indices are displayed for the six control participants and the six patients with Parkinson's disease. There is no overlap between the groups, which are distinct by Student's *t* test ($P < .00005$). The *error bars* represent one SD.

FIG 4. Unified Parkinson's Disease Rating Scale scores for the six patients ranging from 12 to 71 are plotted versus radiologic indices. A linear regression analysis was conducted, yielding a linear correlation coefficient of $r = 0.99$.



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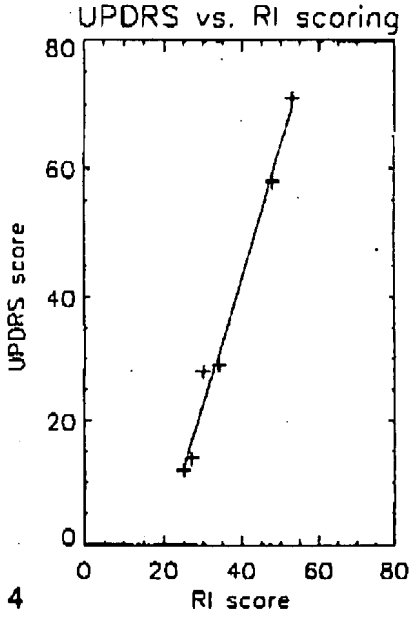

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between radiologic index and Unified Parkinson's Disease Rating Scale score in the patient group ($r = 0.99$).

Discussion

The neuroimaging of the substantia nigra in Parkinson's disease by conventional MR imaging techniques has been a desirable but elusive goal. Potential benefits include the detection of presymptomatic disease as well as the staging of disease. Detection of presymptomatic disease, especially in the inherited disorder, would allow the early introduction of neuroprotective treatments in those determined to be at risk. Furthermore, the potential for staging the disease might allow assessment of neuroprotective interventions in both presymptomatic and symptomatic patients (19). Techniques of this kind may also serve to differentiate idiopathic Parkinson's disease from other forms of parkinsonism.

Visual inspection of the images confirms that the SN_C degenerates from lateral to medial and in an anterior-to-posterior direction. In addition, we note that in all six patients, there were zones of signal loss surrounded by rings of relative preservation in the lower section. These were not seen in any of the control participants. The significance of this particular pattern has yet to be ascertained and corroborated with neuropathologic findings. The sharp delineation of these structures, however, shows that the overall changes observed in the patient group were not the result of motion artifact. Artifacts of this kind tend to blur and degrade small structural changes.

The distinct separation of the patient from the control group in Figure 3 suggests the possibility of detecting presymptomatic disease. (Note that despite the relatively small number of participants in this study, the two groups were distinct at a high level of significance, $P < .00005$). Figure 4 suggests the possibility of staging disease progression with a radiologic index. The correlation between the Unified Parkinson's Disease Rating Scale score and the radiologic index is encouraging, although surprisingly high and probably somewhat fortuitous. As with any clinical scoring system, the Unified Parkinson's Disease Rating Scale values might fluctuate because of the performance of the patient, which can vary from day to day. An imaging technique capable of revealing the severity of the disease has several merits; among other things, it is independent of the physical condition of the patient and of the drugs used to control the symptoms.

Conclusion

A combination of two inversion-recovery sequences has been used to image in vivo the SN_C in healthy participants and in patients with Parkinson's disease. Lateral-to-medial neurodegeneration was confirmed visually in all six patients and allowed for definition of a simple radiologic index to score the severity of the disease. The radiologic index was found to be highly sensitive to even the earliest stages of disease and correlated strongly with the standardized clinical (Unified Parkinson's Disease Rating Scale) score in symptomatic patients. This suggests that MR imaging may not only have the potential for detecting presymptomatic disease but might also be used as a sensitive biological marker for disease progression in both presymptomatic and symptomatic patients. Such a marker could prove invaluable in the assessment of putative neuroprotective therapies. More work is

needed to refine the technique, in particular the use of faster pulse sequences and thinner sections.

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